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### Symposium on 'Micronutrient transport processes'

#### Tocopherol transport and absorption

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Vitamin E is the generic term for a group of lipid-soluble tocol and tocotrienol derivatives possessing varying degrees of vitamin activity. The most active of these compounds is  $\alpha$ -tocopherol, which accounts for approximately 90% of the vitamin E found in tissues. The present paper will, therefore, principally consider the absorption and transport of  $\alpha$ -tocopherol from ingestion to uptake by tissues, including  $\alpha$ -tocopheryl acetate, which is the most widely used form for vitamin E supplementation. Tocopherols are water insoluble, non-swelling amphiphiles. Thus, movement through the aqueous phase at appropriate concentrations depends on the abundance of suitable vehicles enabling efficient vitamin E absorption and transport.

#### ABSORPTION

Vitamin E absorption from the intestinal tract is governed by the same intraluminal membrane and intracellular events as have been described for other dietary fats such as cholesterol or triacylglycerols (Thomson & Dietschy, 1981). The steps include efficient emulsification, solubilization within mixed micelles, diffusion across the unstirred water layer, permeation through the membrane of the absorptive cells (enterocytes), incorporation into lipoprotein particles (chylomicrons), and transport out of the mucosal cell into the circulation via lymphatic pathways.

*Intraluminal events.* Efficient absorption of  $\alpha$ -tocopherol requires the rapid flux of the highly hydrophobic molecule through the thick unstirred water layer coating the absorptive mucosa of the small intestine. This is achieved by the incorporation of  $\alpha$ -tocopherol into mixed bile salt micelles which are formed by the combination of hydrolysed fat (e.g. monoacylglycerols and free fatty acids) and bile salts in the lumen of the small intestine. The aqueous  $\alpha$ -tocopherol concentration can be greatly increased by micellar solubilization and the resulting very small particles can diffuse in substantial amounts across the unstirred water layer to reach the enterocytes. As illustrated in Fig. 1, mixed micelles possess a much greater capacity to solubilize  $\alpha$ -tocopherol and other lipids than do pure bile salt micelles. The mixed micelles have a radius of 1.5–5.0 nm

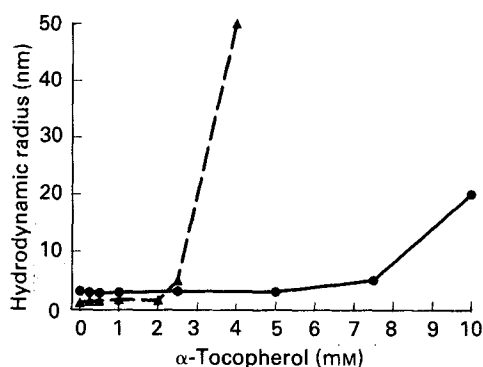


Fig. 1. Mean hydrodynamic radius of  $\alpha$ -tocopherol-containing lipid particles at 37°, measured by quasielastic light scattering. A hydrodynamic radius of <5 nm indicated complete  $\alpha$ -tocopherol solubilization in micelles. (▲), Taurocholate (25 mM) solubilized  $\alpha$ -tocopherol in small micelles of about 1.5 nm radius up to a concentration of 2.5 mM, at higher  $\alpha$ -tocopherol concentrations both a micellar and an oil phase was obtained. Mixed micelles (●) of soya-bean lecithin (12.5 mM), taurocholate (25 mM) and cholesterol (0.3 mM) dissolved up to about 7.5 mM- $\alpha$ -tocopherol, which resulted in mixed micelles of a radius between 2.8 and 5.0 nm. Again, higher  $\alpha$ -tocopherol concentrations resulted in the formation of an oil phase. Micelles were prepared in 30 mM-potassium phosphate buffer (pH 6.7) containing 150 mM-sodium chloride, using the dissolution method of Carey & Small (1978).

(Fig. 1) compared with emulsion particles with a radius of 100–2500 nm (Carey & Small, 1970). Thus, fat digestion involves enzymic processing of the ingested lipids, whereby complex lipids are degraded into components such as monoacylglycerols, as well as physico-chemical events resulting in micellar dispersion of the lipolytic products and  $\alpha$ -tocopherol.

Emulsification begins in the stomach by predominantly mechanical forces which break up large oil droplets into smaller particles. In the intestine chyme mixes with pancreatic and biliary secretions, providing the ingredients necessary for lipid digestion and the formation of mixed bile salt micelles. Triacylglycerols are hydrolysed by pancreatic lipase to monoacylglycerols and fatty acids which are most efficiently dispersed by bile salts. The bile salts are synthesized by the liver and secreted into the small intestine from the gall bladder. The obligatory requirement of bile for the intestinal absorption of vitamin E has been demonstrated in patients with liver diseases and biliary obstruction (Harries & Muller, 1971a; Sokol *et al.* 1987) and in experimental animals (Gallo-Torres, 1970; MacMahon & Thompson, 1970). Additional evidence for the importance of bile salts comes from *in vitro* studies using rat small intestinal slices (Pearson & Legge, 1972) or intestinal cells (Traber *et al.* 1990b). Patients with pancreatic insufficiency, as in cystic fibrosis, secrete greatly decreased amounts of pancreatic enzymes into the intestinal lumen. Such patients also show impaired absorption of  $\alpha$ -tocopherol, presumably caused by a failure to hydrolyse triacylglycerols, thereby resulting in a decreased capacity for  $\alpha$ -tocopherol solubilization in mixed micelles (Bennett & Medwadowski, 1967; Harries & Muller, 1971b; Stead *et al.* 1986). It follows, therefore, that  $\alpha$ -tocopherol absorption is also dependent on the amount and composition of other fats eaten concurrently.

Supplements of vitamin E are generally given in the form of  $\alpha$ -tocopheryl acetate in which the reactive hydroxyl group of  $\alpha$ -tocopherol is esterified, thereby increasing the stability of the molecule. Because essentially all vitamin E is absorbed by the intestinal

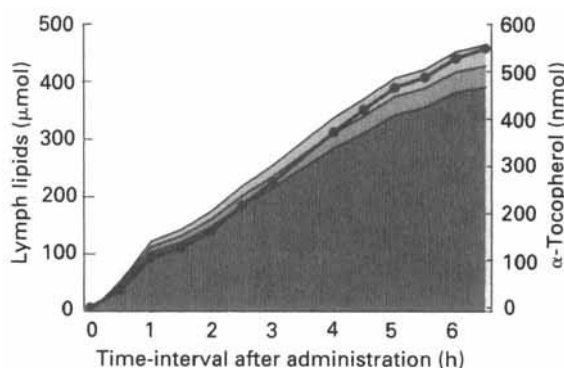


Fig. 2. Cumulative recovery of  $\alpha$ -tocopherol (●) and other lipids in intestinal lymph.  $\alpha$ -Tocopherol (770 nmol) was dispersed in cream (triacylglycerol content 458  $\mu$ mol) and was administered by stomach tube. Rats were prepared as described by Gallo-Torres & Miller (1969). (□), Cholesterol; (⊗), phospholipids; (■), triacylglycerols.

mucosa as free  $\alpha$ -tocopherol,  $\alpha$ -tocopheryl acetate has to be hydrolysed before absorption. The principal enzyme responsible for this hydrolysis is pancreatic esterase (Muller *et al.* 1976), which depends on the presence of bile salts both as an essential cofactor as well as for solubilizing its substrates.

**Uptake by the enterocyte and intracellular events.** Once tocopherol has been solubilized in mixed micelles, transport across the unstirred water layer is facilitated and the vitamin is ready for mucosal uptake by the brush-border membrane. The results of both in vitro and in vivo studies suggest that the rate of vitamin E uptake is controlled by passive diffusion (Hollander *et al.* 1975; Muralidhara & Hollander, 1977). In the rat, maximal absorption takes place at the junction between the upper and middle thirds of the small intestine (Hollander *et al.* 1975; Gallo-Torres, 1980). Within the mucosal cell,  $\alpha$ -tocopherol combines with other lipids and with apoproteins, forming lipoproteins such as the chylomicrons, in which form it is transported out of the cells (Bjorneboe *et al.* 1986; Traber *et al.* 1988). Intracellular  $\alpha$ -tocopherol transport does not appear to rely on a transfer protein (Catignani & Bieri, 1977). Chylomicrons and other lipoproteins containing  $\alpha$ -tocopherol exit the mucosal cells by passing through the basolateral membranes, across the lamina propria, and into the lymphatic stream. After ingestion of  $\alpha$ -tocopherol in cream, about 98% of the vitamin is transported together with the chylomicrons and intestinal very-low-density lipoprotein (VLDL; as defined by its flotation properties), that is with the triacylglycerol-rich lipoproteins. Thus, as illustrated in Fig. 2, the appearance of  $\alpha$ -tocopherol in mesenteric lymph parallels triacylglycerol accumulation. In this experiment the cumulative  $\alpha$ -tocopherol recovery 6.5 h after dosage amounted to about 70% of the dose administered. A similar extent of absorption was found by Traber *et al.* (1986). However, variable recoveries of  $\alpha$ -tocopherol in lymph have been reported following cannulation of the thoracic duct in rats (Gallo-Torres, 1980). Depending on the form and formulation tested, the extent of absorption varied between 10 and 65%, probably reflecting differences during the intraluminal phase of absorption. Although there is evidence that some of the  $\alpha$ -tocopherol may be taken up by the portal system (MacMahon *et al.* 1971; Hollander & Dadufalza, 1989), at least the majority of the absorbed tocopherol is carried via the lymphatics to the bloodstream.

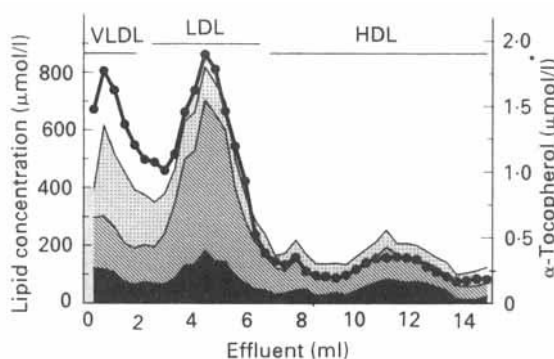


Fig. 3. Distribution of  $\alpha$ -tocopherol (●) among human plasma lipoproteins. Lipoproteins were separated by gradient ultracentrifugation (Cohn & Kuhn, 1989). The plasma concentrations of the major lipid components in the lipoprotein fractions are shown. (▨), Triacylglycerols; (▩), cholesterol; (■), phospholipids.

#### PLASMA TRANSPORT AND TISSUE DELIVERY

Vitamin E is transported in the plasma by the plasma lipoproteins and there is no evidence of a specific plasma carrier protein as has been found for vitamins A and D (Bjornson *et al.* 1976; Behrens *et al.* 1982). In consequence, co-transport of  $\alpha$ -tocopherol with other circulating lipids ensures protection of polyunsaturated fatty acids from free radical attack (Esterbauer *et al.* 1987). Moreover, plasma vitamin E concentrations do not entirely depend on dietary intake, but vary with the lipoprotein concentrations (Davies *et al.* 1969; Horwitt *et al.* 1972). Normal plasma  $\alpha$ -tocopherol concentrations range from 11–37  $\mu\text{mol/l}$  (Farrell, 1980).

**Lipoprotein distribution.** All the lipoprotein fractions contain  $\alpha$ -tocopherol, and its distribution parallels the distribution of lipids in lipoproteins, as shown in Fig. 3. In the human, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) represent the most abundant lipoprotein classes and are the major carriers of  $\alpha$ -tocopherol. As pointed out by Behrens *et al.* (1982), more  $\alpha$ -tocopherol is found in HDL than in LDL in females, whereas in males the opposite is true. The distribution of  $\alpha$ -tocopherol in lipoproteins does not parallel individual lipid classes such as triacylglycerols, cholesterol or phospholipids (Fig. 3), indicating that the vitamin does not share the same metabolic fate as any one of these lipids. Furthermore, the lipoprotein distribution of  $\alpha$ -tocopherol does not reflect the relative importance of individual lipoprotein classes for the transport of vitamin E. For example, as discussed earlier, tocopherol enters the systemic circulation within chylomicrons, which in terms of turnover are the major carriers of vitamin E. However, as the secretion of newly synthesized chylomicrons fluctuates with the intake of dietary lipids, and clearance of this lipoprotein is fast, plasma concentrations of vitamin E associated with the chylomicron fraction are usually low. Thus, the distribution of vitamin E in the plasma lipoproteins represents a steady-state which is adjusted by various metabolic processes, as discussed later (p. 185).

**Redistribution during lipoprotein metabolism.** The fat transport system can be divided into two pathways: an exogenous pathway for dietary lipids absorbed from the intestine and an endogenous pathway for lipids entering the bloodstream from the liver and other non-intestinal tissues.

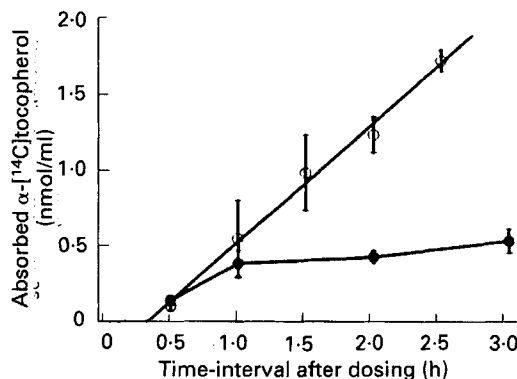


Fig. 4. Effect of Triton WR 1339 on the appearance of absorbed  $\alpha$ - $[^{14}\text{C}]$ tocopherol in rat plasma. Labelled  $\alpha$ -tocopherol ( $0.93 \mu\text{mol}$ ) was dispersed in cream ( $2 \text{ ml}$ ) at a specific activity of  $22.63 \mu\text{Ci}/\text{mmol}$  and administered by stomach tube to Triton-treated ( $\circ$ ) and control ( $\bullet$ ) rats ( $n 3$ ). For Triton treatment, rats were injected with the detergent  $30 \text{ min}$  before the ingestion of the labelled tocopherol (Cohn *et al.* 1988). Values are means and one standard deviation expressed as  $\alpha$ - $[^{14}\text{C}]$ tocopherol concentrations, since plasma radioactivity was shown to represent  $\alpha$ -tocopherol exclusively.

*The exogenous pathway.* Following its absorption, vitamin E enters the circulation by means of chylomicrons and intestinal VLDL. In order to monitor the appearance of these intestinal lipoproteins, the intravascular metabolism of chylomicrons and VLDL has been blocked by injection of Triton WR 1339 (Otway & Robinson, 1967*a,b*). Triton inhibits the clearance of chylomicrons and VLDL (Risser *et al.* 1978; Holt & Dominguez, 1980), rendering these lipoproteins inaccessible for hydrolysis by lipoprotein lipase (*EC* 3.1.1.34), an enzyme situated on the capillary endothelial surface of adipose tissue and muscle (Nelsson-Ehle *et al.* 1980).

The effect of Triton on the appearance of ingested  $\alpha$ - $[^{14}\text{C}]$ tocopherol in rat plasma is shown in Fig. 4. Approximately  $30 \text{ min}$  after its administration,  $\alpha$ - $[^{14}\text{C}]$ tocopherol could be detected in plasma and, thereafter, the label accumulated linearly with time. Without Triton injection, plasma concentrations of absorbed  $\alpha$ - $[^{14}\text{C}]$ tocopherol in the control rats were much lower and mainly increased between  $30$  and  $60 \text{ min}$  after dosage. This result indicated that the lower vitamin E concentrations found in the control rats were due to efficient removal of the vitamin from the plasma and its transfer to tissues. Accordingly,  $150 \text{ min}$  after administration of  $\alpha$ - $[^{14}\text{C}]$ tocopherol recovery from the liver was  $4\%$  of the dose in the controls, but only  $0.3\%$  in Triton-treated rats. Following Triton injection, ingested  $\alpha$ - $[^{14}\text{C}]$ tocopherol accumulated mainly within chylomicrons (Fig. 5(a)), the remaining plasma  $\alpha$ -tocopherol being confined to the VLDL fraction. These findings were in contrast to the post-prandial alterations in lipoprotein  $\alpha$ -tocopherol content of non-Triton control rats (Fig. 5(b)), where only small amounts of  $\alpha$ - $[^{14}\text{C}]$ tocopherol were associated with VLDL and chylomicrons. The major portion of the  $\alpha$ -tocopherol accumulated within the density  $1.006$  infranate, comprising chylomicron remnants, intermediate-density lipoprotein (IDL), LDL and HDL. Thus, dietary  $\alpha$ -tocopherol enters plasma on chylomicrons and VLDL and during their catabolism by lipoprotein lipase,  $\alpha$ -tocopherol is subsequently transferred to remnants, LDL and HDL.

These results supplement the findings of Bjorneboe *et al.* (1986) who injected  $\alpha$ - $[^3\text{H}]$ tocopherol-labelled lymph into rats. The  $\alpha$ - $[^3\text{H}]$ tocopherol was cleared from the circulation with a half-life of about  $12 \text{ min}$ , which is in agreement with the half-lives

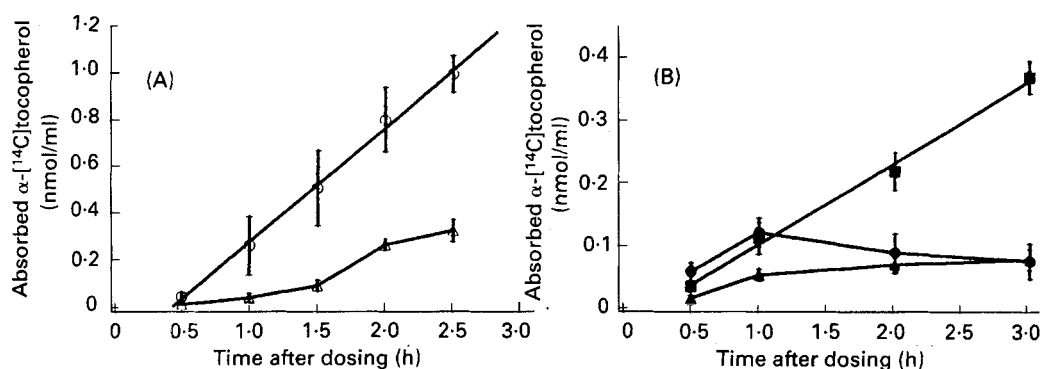


Fig. 5. Effect of Triton WR 1339 on the appearance of absorbed  $\alpha$ -[ $^{14}$ C]tocopherol in plasma lipoprotein fractions. (A), Triton-treated rats; (B), control rats. Lipoproteins were separated as described (Cohn *et al.* 1988). Labelled  $\alpha$ -tocopherol ( $0.93 \mu\text{mol}$ ) was dispersed in cream (2 ml) at a specific density of  $22.63 \mu\text{Ci}/\text{mmol}$  and administered by stomach tube to the rats. For Triton treatment, rats were injected with the detergent 30 min before the ingestion of the labelled tocopherol (Cohn *et al.* 1988). (○, ●), Chylomicrons; (△, ▲), very-low-density lipoprotein; (■), density  $>1.006$  infranate. Values are means and one standard deviation, expressed as  $\alpha$ -[ $^{14}$ C]tocopherol concentrations, since plasma radioactivity was shown to represent  $\alpha$ -tocopherol exclusively.

reported for other chylomicron constituents such as retinyl palmitate. At 10 min after intravenous injection of chylomicrons, about 8% of the  $\alpha$ -[ $^3\text{H}$ ]tocopherol was recovered in the HDL fraction. The labelled  $\alpha$ -tocopherol was preferentially taken up by the liver, which after 1 h contained more than 50% of the dose, most of the tracer being recovered in parenchymal cells. As discussed later (p. 184), these findings are consistent with a preferential  $\alpha$ -tocopherol plasma clearance in chylomicron remnants via the apo E receptor pathway.

Efficient removal of newly absorbed  $\alpha$ -tocopherol from the circulation depends, therefore, on the action of lipoprotein lipase. Under the influence of this enzyme much of the  $\alpha$ -tocopherol is taken up by the liver or transferred to other lipoproteins. In addition, Traber *et al.* (1985) demonstrated that lipoprotein lipase transfers  $\alpha$ -tocopherol from chylomicrons to fibroblasts in cell culture during triacylglycerol hydrolysis. Tissues capable of synthesizing and secreting lipoprotein lipase, such as adipose tissue and muscle, may, therefore, obtain tocopherol by the lipoprotein lipase mechanism. During intravascular hydrolysis of triacylglycerols by lipoprotein lipase, some of the core material from the chylomicrons or VLDL is removed; consequently, less surface material consisting of unesterified cholesterol, phospholipids and apolipoproteins is required (Havel, 1987). This redundant material moves along a surface pathway to HDL, and apolipoprotein E moves from HDL to chylomicrons or VLDL. Some tocopherol is probably transferred with the excess surface material from chylomicrons and VLDL to HDL during the hydrolysis of triacylglycerols. Lipoprotein lipase releases remnant particles containing the remaining core lipids (cholesteryl esters, triacylglycerols, vitamin E, etc.) which are cleared from the circulation by the liver via the apo E receptor pathway (Mahley, 1988).

*The endogenous pathway.* The endogenous pathway begins with the hepatic secretion of VLDL. A number of different *in vitro* and *in vivo* studies have shown that  $\alpha$ -tocopherol is secreted into the bloodstream within nascent VLDL (Bjorneboe *et al.*



1987; Cohn *et al.* 1988). Since the delivery of dietary  $\alpha$ -tocopherol by chylomicrons fluctuates with the load of absorbed vitamin E, hepatic VLDL and the endogenous pathway are important for maintaining plasma concentrations of  $\alpha$ -tocopherol. Using deuterated tocopherols, the liver was found to secrete nascent VLDL which were enriched in natural  $\alpha$ -tocopherol preferentially to  $\gamma$ -tocopherol and the other stereoisomers of  $\alpha$ -tocopherol (Traber *et al.* 1990a,c,d). This biodiscrimination appears to be related to an intracellular tocopherol-binding protein (31 kD), which exhibits different binding affinities for the various vitamin E homologues and stereoisomers (Catignani & Bieri, 1977; Behrens & Madere, 1982).

Similarly as described for chylomicrons and intestinal VLDL, hepatic VLDL are also metabolized in peripheral tissues by lipoprotein lipase. This results in the formation of IDL and eventually LDL. Again lipoprotein lipase could promote the delivery of VLDL- $\alpha$ -tocopherol to adipose tissue and muscle as well as mediate  $\alpha$ -tocopherol transfer to HDL along with other excess surface material of VLDL. Transfer of tocopherol from HDL to other lipoproteins could then occur enriching all the lipoprotein fractions with the vitamin.  $\alpha$ -Tocopherol has been shown to exchange spontaneously between different lipoproteins (Massey, 1984). Transfer is efficient from LDL and HDL to other lipoproteins, whereas VLDL and chylomicrons are comparatively poor  $\alpha$ -tocopherol donors (Granot *et al.* 1988; W. Cohn, unpublished results). The transfer of tocopherol between lipoproteins is not assisted by the neutral-lipid transfer protein, which promotes the exchange of cholesteryl ester for triacylglycerol between HDL and the triacylglycerol-rich lipoproteins (Granot *et al.* 1988).

The IDL and LDL produced by the metabolism of hepatic VLDL can be taken up both by the liver and by peripheral tissues via LDL (apolipoprotein B/E) receptors (Gotto *et al.* 1986). The high-affinity receptor pathway for LDL has been shown to function as a mechanism for  $\alpha$ -tocopherol delivery to fibroblasts in tissue culture (Traber & Kayden, 1984; Thellman & Shireman, 1985). Consistent with endocytosis of intact LDL, both  $\alpha$ -tocopherol and apo B, the apolipoprotein of LDL, were taken up together in these studies. When LDL was incubated with receptor-negative fibroblasts from a patient with homozygous familial hypercholesterolaemia, delivery of  $\alpha$ -tocopherol was reduced. Uptake of both  $\alpha$ -[ $^3$ H]tocopherol and  $^{125}$ I-labelled LDL by normal fibroblasts was greatly reduced when the lipoprotein was modified by methylation, which prevented binding of LDL to its receptor (Weisgraber *et al.* 1978). The residual uptake of  $\alpha$ -tocopherol from either methylated LDL by normal cells, or from normal LDL by LDL-receptor-deficient cells, suggest that the LDL-receptor mechanism is not the only pathway for delivery of tocopherols from LDL to cells. This is consistent with the observation that patients with the homozygous form of familial hypercholesterolaemia do not manifest any biochemical or clinical evidence of vitamin E deficiency.

The role of the LDL-receptor pathway in the maintenance of  $\alpha$ -tocopherol concentrations in tissues of intact animals has been further investigated in the Watanabe heritable hyperlipidaemic (WHHL) rabbit (Cohn & Kuhn, 1989). These animals have a defect in the intracellular glycosylation of the immature LDL receptor, which causes decreased transport of the receptor to the cell surface and a virtual absence of LDL-receptor activity (Yamamoto *et al.* 1986). Thus, WHHL rabbits provide an animal model for homozygous familial hypercholesterolaemia. Plasma concentrations of  $\alpha$ -tocopherol in WHHL rabbits fed on a control diet for 200 d were increased tenfold compared with control rabbits, and this was associated with increased concentrations of

VLDL, IDL and LDL. Despite the LDL-receptor deficiency in the WHHL animals, tissue concentrations of  $\alpha$ -tocopherol, with the exception of the adrenal gland, were not reduced. The adrenal gland, thus, appears to rely on the LDL-receptor pathway for obtaining a significant proportion of its  $\alpha$ -tocopherol. Following the injection of LDL labelled with  $\alpha$ -[ $^3\text{H}$ ]tocopherol into both normal and WHHL rabbits, there was evidence for transfer of  $\alpha$ -[ $^3\text{H}$ ]tocopherol to tissues independent of LDL uptake (Cohn *et al.* 1992). In addition,  $\alpha$ -[ $^3\text{H}$ ]tocopherol was found to move between the various lipoprotein fractions. The results also indicated that the high concentrations of VLDL, IDL and LDL in WHHL rabbits allow more efficient clearance by receptor-independent mechanisms. Taken together, these findings show that LDL- $\alpha$ -tocopherol may be delivered to tissues by a number of different mechanisms, including uptake of  $\alpha$ -tocopherol with LDL by receptor-dependent and -independent pathways, and uptake of  $\alpha$ -tocopherol without concomitant LDL uptake. Moreover,  $\alpha$ -tocopherol can exchange from LDL to other lipoproteins, which in turn can mediate transfer of  $\alpha$ -tocopherol to tissues.

Little is known about HDL-mediated tissue uptake of vitamin E. HDL-binding sites recognizing apolipoproteins A-I and A-II have been identified on cell surfaces. These binding sites are involved in cholesterol efflux from cells to HDL (Oram *et al.* 1983; Aviram *et al.* 1989; Tozuka & Fidge, 1989). In current studies (W. Cohn, unpublished results) the liver was the predominant site of HDL- $\alpha$ -tocopherol uptake, consistent with a role for HDL in reverse  $\alpha$ -tocopherol transport from tissues to the liver. The adrenal gland was the most active tissue per unit wet weight for tocopherol uptake. At 15 min after injection of labelled HDL, more than 40% of the  $\alpha$ -tocopherol tracer was recovered in the liver. Studies with primary cultures of rat hepatocytes confirmed that the transfer from HDL to cells was fast and efficient.

#### CONCLUSION

The concepts presented here reflect mainly the progress made in the understanding of vitamin E plasma transport during the last decade. However, many aspects of vitamin E absorption, allocation to lipoproteins and tissue delivery remain largely unknown. For example, we still ignore how  $\alpha$ -tocopherol is transported from the brush-border membrane of the enterocyte to the golgi complex for incorporation into chylomicrons. The question of whether transfer factors are involved in the distribution of  $\alpha$ -tocopherol within lipoproteins and its movement to tissues remains to be settled.

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